REMARKS

Claims 2-15 and 46-51 are pending in this application. Claims 2 and 47 have been amended herein to correct a typographical error so that the claims recite "neural precursor" cells instead of "neuronal precursor" cells. Support for this amendment is found in the specification at, *inter alia*, page 7, lines 15-17. Claims 2, 6, 8, 13, 15, and 47 have been amended herein to more clearly claim the invention. Support for these amendments is found, *inter alia*, in the claims as originally filed and generally throughout the specification. Accordingly, no new matter has been added by these amendments.

Claims 4, 5, 7, 14, 49, and 51 have been cancelled herein without prejudice or disclaimer of the subject matter claimed therein for prosecution at a later date.

New claims 52-75 have been added herein. Support for these new claims is found, *inter alia*, in the claims as originally filed, and in the specification at page 24, line 20 to page 26, line 33. Accordingly, no new matter has been added by these new claims.

Applicant notes that the Office Action Summary does not list claim 46 as pending in the application. Applicants respectfully submit that claim 46 is pending in the application and was included the rejections of the claims.

Therefore, after entry of these amendments claims 2-3, 6, 8-13, 15, 46-48, 50, and 52-75 will be pending in the application.

The specification has been amended to correct various obvious typographical errors. Accordingly, no new matter has been added by these amendments.

Applicant would further like to clarify on the record that in response to the rejection of claims 1-15 and 46 under 35 U.S.C. § 112, second paragraph, in the previous Office Action, Applicant did not merely cancel the phrase "neuronal and glial properties." Applicant replaced this phrase with the phrase "<u>have the ability to differentiate to neuronal or glial cells</u>." Applicant intended to traverse this rejection. However, in order to expedite prosecution, Applicant replaced the allegedly indefinite language with similar language hopefully acceptable to the Examiner. As this rejection

was not repeated in the current Office Action and the Examiner has indicated that rejections and objections not reiterated from the previous Office Action are withdrawn, Applicant assumes that this language was acceptable.

The outstanding rejections are addressed individually below.

1. The claims are enabled by the specification as filed.

Claims 2-15 and 46-51 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to provide an enabling disclosure for the claimed cell composition. Applicant respectfully traverses this rejection.

As claims 4, 5, 7, 14, 49, and 51 have been cancelled in this Amendment, Applicant respectfully submits that the rejection has been rendered moot as to the cancelled claims.

The Office Action states that while being enabling for a composition comprising up to 66% neural precursor cells derived from ES cells, the application does not reasonably provide enablement for a composition comprising from 85% to 100% isolated neural precursor cells as recited in the claims. Applicant respectfully disagrees.

The 66 ± 3% value referred to in the specification at page 24, lines 33-38, refers to the fraction of nestin-positive cells detectable five days after plating of ES cell-derived spheres in the absence of growth factors. In these conditions, the ES cell-derived spheres are induced to differentiate. Accordingly, the cell composition at this time point also includes approximately 34% neurons, 30% astrocytes and 6% oligodendrocytes. The percentages add up to greater than 100% because many of the immature GFAP-positive astrocytes still coexpress nestin.

However, the 66% value does <u>not</u> refer to the maximum number of neural precursor cells obtainable in the composition of the present invention. The fraction of neural precursor cells obtained by the method in Example 2 and in Example 3 typically approaches 100%.

This fact is corroborated by the Declaration of Dr. Bruestle under 37 C.F.R. § 1.132[1] submitted herewith, which provides data indicating that the undifferentiated, tumorigenic ES cells have been essentially eliminated from the cell compositions of the present invention, and that neural precursor cells have been generated in unprecedented purity approaching 100%. The Declaration of Dr. Bruestle indicates that among other indications regarding the purity of the neural precursor cells, experiments were performed in which a cocktail of antibodies was applied to markers of both immature (nestin and A2B5) and differentiated (beta-III-tubulin, GFAP, and 04) neural precursor cells in order to determine the overall purity of the generated neural cells. The data demonstrates that the cell compositions of the present invention contain more than 99% neural cells. (Declaration of Dr. Bruestle, paragraph 17) This higher purity is necessary in order to obtain the non-tumorigenic cell compositions of the present invention.

Accordingly, Applicant respectfully requests that this § 112, first paragraph, rejection be reconsidered and withdrawn.

2. ***Amended claims are definite.***

Claims 2-15 and 46-51 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite in their recitation of "neuronal precursor cells, which have the ability to differentiate to neuronal or glial cells."

As claims 4, 5, 7, 14, 49, and 51 have been cancelled in this Amendment, Applicant respectfully submits that the rejection has been rendered moot as to the cancelled claims.

Claims 2 and 47 have been amended herein as suggested by the Examiner to recite "neural precursor cells." It is obvious from the specification that this was a typographical error as the specification indicates at page 7, lines 15-17 that neural precursor cells are "[i]mmature cell[s] of the nervous system which [have] the potential

[1] This Declaration is being submitted unsigned. The executed version will be submitted as soon as possible.

to develop into mature nervous system cells such as neurons and glia (astrocytes and oligodendrocytes)" and the claim clearly indicates that the intended cells have the ability to differentiate to "neuronal cells, glial cells, or combinations thereof."

Therefore, in view of the amendment, Applicant respectfully requests that this § 112, second paragraph, rejection be reconsidered and withdrawn.

3. The claims are novel.

Claims 2-15 and 46-51 stand rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Okabe *et al.* (1996). Applicant respectfully traverses this rejection.

As claims 4, 5, 7, 14, 49, and 51 have been cancelled in this Amendment, Applicant respectfully submits that the rejection has been rendered moot as to the cancelled claims.

Okabe *et al.* teaches a method for differentiation of neuroepithelial precursor cells. The cells were cultivated in medium containing ITSFn and were subsequently proliferated in the presence of basic fibroblast growth factor (bFGF). By withdrawal of bFGF, some of the cells differentiated into neurons and glia. The cultures obtained by the described method contain merely up to 84.1% nestin positive cells. (See Okabe *et al.*, Table 1).

Nestin is a protein marker for precursor cells of neurons, astrocytes and oligodendrocytes. However, nestin is also expressed in mature glia cells as well as in non-neural cells, such as endothelial cells and hematopoietic cells. (*See* Sotelo *et al.*, *J. Neurosci.*, 14(1):124-133, 1994, Attached hereto as Appendix A)

Thus, the expression of nestin is not sufficient for determining the purity of a neuroepithelial cell population. The cell composition disclosed in Okabe *et al.* contains up to 84.1% nestin positive cells. Applicant notes that the reference at page 97, second column, last sentence indicates that up to 95% of the cells are stained with the neuroepithelial precursor cell marker nestin; however, data indicating these results is not shown. It should be noted, however, that these cells will not necessarily become

neural precursor cells. The ratio of glia cells, endothelial cells and hematopoietic cells in the nestin-positive population is not determined in the reference. Therefore, it is not possible to draw conclusions concerning the purity of the cell population in the Okabe *et al.* paper.

By comparison, the present invention discloses a method for the production of non-tumorigenic precursor cells, wherein the precursor cells are obtained from embryonic stem cells and contain from 0% to 15% primitive embryonic and non-neural cells.

After transplantation into the nervous system, undifferentiated embryonic stem cells may develop into tumors and non-neural differentiated cells may form non-neural tissue in transplants.

As described in the Declaration of Dr. Bruestle under 37 C.F.R. § 1.132, further studies of the Applicant as well as independent third persons have shown that embryonic stem cells that have been cultured according to the Okabe *et al.* method caused tumor development after transplantation into the active nervous system.

In Dr. Bruestle's declaration, he notes and provides data demonstrating that cell populations generated by the method of Okabe *et al.* still contain a significant fraction of undifferentiated alkaline phosphatase-positive cells, in contrast to cells subsequently propagated in the presence of EGF+FGF2 or EGF+FGF2 followed by FGF2+PDGF. These cells contain no alkaline phosphatase-positive colonies. Accordingly, the presence of alkaline phosphatase-positive cells correlates with the tumorigenicity of cells generated by the Okabe method. (Declaration of Dr. Bruestle, paragraphs 9-10)

The Declaration of Dr. Bruestle also refers to a statement of Dr. Bengzon (attached to the Declaration of Dr. Bruestle as Appendix D) which discloses that when transplanted into the rat brain, neural precursor cells produced according to the protocol later published in Okabe *et al.* consistently gave rise to non-neural adenoid tissue and tumors. (Declaration of Dr. Bruestle, paragraph 10 and Appendix D)

Additionally, Wernig *et al.*, a manuscript that has been submitted for publication (attached to the Declaration of Dr. Bruestle as Appendix G), also states that "[t]hese and earlier findings . . . indicate that ES cell-derived neural precursors generated according to the protocol of Okabe et al. (1996) still contain a fraction of undifferentiated cells capable of teratoma formation." (Wernig *et al.*, page 18)

Thus, the compositions of the Okabe *et al.* reference are not non-tumorigenic neural precursor cells, as required by Applicant's claims.

In summary, Okabe *et al.* does not disclose the production of a non-tumorigenic cell composition derived from embryonic stem cells which comprises from 85% to 100% isolated neural precursor cells, which have the ability to differentiate into neuronal cells, glial cells or combinations thereof, and further comprising from 0% to 15% primitive embryonic and non-neural cells.

Thus, as these claims are not anticipated by Okabe *et al.*, Applicant respectfully requests that their rejection under 35 U.S.C. § 102(b) be reconsidered and withdrawn.

CONCLUSIONS

In view of the arguments set forth above, Applicant respectfully submits that the rejections contained in the final Office Action mailed on February 26, 2003, have been overcome, and that the claims are in condition for allowance. If the Examiner believes that any further discussion of this communication would be helpful, she is invited to contact the undersigned at the telephone number provided below.

Applicant encloses herewith a Petition for a Three Month Extension of Time pursuant to 37 C.F.R. § 1.136 up to and including August 26, 2003, to respond to the Examiner's Office Action mailed on February 26, 2003. Please charge deposit account no. 08-0219 the $465.00 fee (small entity) for this purpose.

Applicant also encloses herewith a Notice of Appeal. Please charge deposit account no. 08-0219 the $160.00 fee (small entity) for this purpose.

Applicant also encloses herewith a Supplemental Information Disclosure Statement. Please charge deposit account no. 08-0219 the $180.00 fee for this purpose.

No other fees are believed to be due in connection with this response. However, please charge any underpayments or credit any overpayments to Deposit Account No. 08-0219.

Respectfully submitted,

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Attachment: Reference cited in response (Sotelo *et al.*, *J. Neurosci.*, 14(1):124-133, 1994)